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Cytembena: Condensation of the Carcinogenesis Bioassay Technical Report*

Cytembena (cytembene; 2-butenoic acid; 3-bromo-4(4-methoxyphenyl)-4-oxo-, sodium; NSC 104801; CAS No. 21739-91-3) was selected for testing by the Carcinogenesis Testing Program, National Cancer Institute (now part of the National Institute of Environmental Health Sciences/National Toxicology Program) because preliminary clinical results from Czechoslovakia indicated that this chemical might have use as a cytostatic agent (*1-4*). Clinical trials in the United States were discontinued because no apparent antineoplastic effects were seen (*5-7*).

Methods

Male and female inbred Fischer 344 rats and male and female hybrid $B6C3F_1$ mice, obtained from Frederick Cancer Research Center, were used in this study. Control and treated groups comprised 50 animals of each species and sex. For 104 consecutive weeks all groups were fed Wayne Lab Blox. Three times per week treated groups were given intraperitoneal injections of 7 or 14 mg/kg (for rats) and 12 or 24 mg/kg (for mice) of cytembena (99.4% pure). Control groups received 0.9% saline injections.

This carcinogenesis bioassay was conducted during January 1977 to January 1979 by Southern Research Institute under a subcontract to Tracor Jitco (prime contractor for the testing program).

All animals that died during the study or that were killed at the end of the exposure period were subjected to a gross necropsy and a complete histopathological examination. Statistical analyses comparing survival and numbers of animals with specific site tumors were done with trend tests and pairwise comparisons (*8-11*). The study design conformed to the NCI Guidelines for carcinogen bioassay (*12*).

Results

Mean body weights of dosed and vehicle-control rats were comparable throughout the bioassay. Mean body weights of dosed and vehicle-control mice were comparable for the first 73 weeks of the bioassay; mean body weight of the high dose male mice was slightly lower than that of the vehicle controls after 73 weeks, and that of the high dose female mice was lower after week 87.

Survival for male rats was significantly decreased ($p < 0.001$) in the dosed groups relative to controls (29/50 control, 12/50 low dose, 12/50 high dose), whereas survival rates for female rats were comparable (30/50, 33/50, 28/50). All male mice groups exhibited markedly decreased survival compared to historic rates (20/50, 19/50, 12/50); female mice survival was comparable among groups (41/50, 33/50, 34/50).

Increased tumor incidences were diagnosed in male rats for mesotheliomas and in female rats for mammary gland fibroadenomas (Table 1).

The numbers of cytembena-treated rats with other specific site tumors and the frequencies of cytembena-treated mice with lesions did not differ significantly from those observed in controls. Tables 2 (rats) and 3 (mice) list those primary tumors found in at least three animals of any one group.

Discussion

The increased number of treated male rats with mesotheliomas in the tunica vaginalis and in multiple organs clearly associates these lesions with cytembena administration (Table 1). Malignant

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Single copies of the complete technical report (TR 207) may be obtained from the Public Information Office, National Toxicology Program, P. O. Box 12233, Research Triangle Park, North Carolina 27709.

Table 1. Primary tumor increases in male and female F344 rats given cytembena by intraperitoneal injection.

Tumor	Male			Female		
	Vehicle control	7 mg/kg	14 mg/kg	Vehicle control	7 mg/kg	14 mg/kg
Mammary gland fibroadenomas[a]	0/50	2/50	2/50	13/49	22/50	36/50[b]
Mesothelioma mesentery	0/50	3/50	0/50	0/49	0/50	0/50
Mesothelioma multiple organs[c]	3/50	26/50[b]	26/50[b]	0/49	0/50	2/50
Mesothelioma tunica vaginalis[d]	0/50	11/50[b]	10/50[e]	–	–	–

[a]Significant dose-related trend for females ($p < 0.001$).
[b]Significantly greater than controls ($p < 0.001$).
[c]Significant dose-related trend for males ($p < 0.001$).
[d]Significant dose-related trend for males ($p < 0.05$).
[e]Significantly greater than controls ($p < 0.005$).

Table 2. Primary tumors in male and female F344 rats given cytembena by intraperitoneal injection.

Tumor	Male			Female		
	Vehicle control	7 mg/kg	14 mg/kg	Vehicle control	7 mg/kg	14 mg/kg
Adrenal pheochromocytoma	12/50	7/50	7/49	3/49	2/50	1/50
Hepatocellular neoplastic nodule	1/49	1/50	1/50[a]	0/49	1/50	4/50
Leukemia	20/50	12/50	22/50	8/49	8/50	8/50
Lymphoma	0/50	0/50	0/50	0/49	3/50	1/50
Mesentery lipoma	0/50	2/50	2/50	0/49	0/50	3/50[b]
Pancreatic islet-cell adenoma or carcinoma	3/50	5/50	4/49	0/49	1/48	1/49
Pituitary adenoma	8/50	3/50	5/50	14/47	13/46	16/47
Subcutaneous fibroma or fibroadenoma	3/50	0/50	2/50	0/49	0/50	2/50
Testicular interstitial cell	47/50	41/50	47/50	–	–	–
Thyroid C-cell adenoma or carcinoma	5/50	4/49	4/50	8/49	6/48	8/49
Thyroid follicular-cell adenoma or carcinoma	3/50	1/49	3/50	1/49	2/48	2/49
Uterine endometrial stromal polyp	–	–	–	16/49	11/50	11/50

[a]One other male rat had a hepatocellular carcinoma.
[b]One other female rat had a sarcoma, NOS (not otherwise specified).

Table 3. Primary tumors in male and female $B6C3F_1$ mice given cytembena by intraperitoneal injection.

Tumor	Male			Female		
	Vehicle control	12 mg/kg	24 mg/kg	Vehicle control	12 mg/kg	24 mg/kg
Circulatory system hemangiosarcoma	1/50	3/50	1/50	0/50	1/50	2/50
Harderian gland adenoma	2/50	3/50	1/50	1/50	0/50	1/50
Hepatocellular adenoma	6/49	5/49	6/50	0/50	0/48	4/49
Hepatocellular carcinoma	10/49	13/49	7/50	3/50	3/48	2/49
Lymphoma	5/50	4/50	3/50[a]	8/50	8/50[a]	11/50[b]
Lung alveolar/bronchiolar adenoma or carcinoma	6/50	7/47	5/49	7/50	4/49	2/48
Pituitary adenoma	0/47	0/42	0/44	1/47	3/46	0/44
Pituitary carcinoma	0/47	0/42	0/44	1/47	0/46	1/44
Thyroid follicular-cell adenoma	1/44	0/44	2/46	3/49[c]	2/48	2/49[c]
Uterine endometrial stromal polyp or sarcoma	–	–	–	0/49	3/48	2/50

[a]One other animal had leukemia.
[b]Two other females had leukemia.
[c]One other female had a thyroid follicular-cell carcinoma.

mesotheliomas involved the serosal surfaces of most of the abdominal organs including the spleen, pancreas, mesentery, urinary bladder, seminal vesicles, prostate, testicles (tunica vaginalis and epididymis), peritoneum, abdominal wall, and diaphragm. Grossly, abdominal distention was frequently observed. This distention resulted from accumulation of large quantities of dark brown peritoneal fluid. Most serous surfaces manifested a rough, granular appearance. The mesentery was usually thickened and nodular.

Histologically, most mesotheliomas were of the papillary type. There were numerous villous or papillary projections on the serous surface of affected organs. The stromal element of the villi consisted of connective tissue cells. The villi or projections were covered by prominent mesothelial cells. The cell nuclei had moderately dense stippled chromatin and were uniformly round to oval. Most nuclei had one or two small nucleoli. The cytoplasm was distinct and abundant. Mitotic figures were commonly seen. There was frequent invasion of the abdominal wall and diaphragm. Invasive cells were usually cuboidal or polygonal and sometimes formed pseudoglandular acini. These cells were usually more pleomorphic and were arranged in solid nests or cords.

Cytembena induced an increased incidence of female rats with proliferative and neoplastic lesions of the mammary gland. A high incidence of atypical mammary gland fibroadenomas was observed in the female rats. These neoplasms were characterized by numerous cystic ducts with extensive periductular fibrosis and cystic glandular hyperplasia. Many of the ducts contained numerous intraductular papillary growths. Epithelial cells covering the papillary projections often showed piling up of cell nuclei. In some areas, there were dilated periductular acini. The epithelial cells lining these spaces stained deeply basophilic and frequently showed a loss of normal cellular orientation or polarity. Some mitotic activity was observed in these areas. The variable degrees of cellular atypia were suggestive of early malignant transformation or change. These lesions represented a variant of mammary fibroadenomas. There was also an increase in the number of cystic ducts, cystic hyperplasia, epithelial hyperplasia, and lobular hyperplasia in the mammary tissue of dosed female F344 rats.

Positive dose-related trends were observed for female rats with hepatocellular neoplastic nodules ($p < 0.05$) and for female rats with mesentery lipoma ($p < 0.05$). Pairwise comparisons between treated and control groups did not yield any significant differences. These borderline effects were not considered compound induced. Similarly, a significant trend ($p < 0.05$) was calculated for female mice with hepatocellular adenoma; no differences were found between treated groups and controls or when liver tumors (adenomas and carcinomas) were considered together.

In conclusion, under the conditions of this bioassay, cytembena was carcinogenic for male and female F344 rats, causing increased numbers of males with mesotheliomas in the tunica vaginalis and in multiple organs and causing increased frequencies of females with fibroadenomas in the mammary glands. Cytembena was not carcinogenic for $B6C3F_1$ mice of either sex (*13*).

REFERENCES

1. Dvorak, O., Venta, J., and Semonsky, M. Report on treatment of advanced carcinoma of genitals with the preparation MBBA. Neoplasma 12(1): 93-99 (1965).
2. Dvorak, O. Cytembena treatment of advanced gynaecological carcinomas. Neoplasma 18(5): 461-464 (1971).
3. Dvorak, O., and Bauer, J. Cytembena treatment of advanced and relapsing uterine cervic carcinoma. Neoplasma 18(5): 465-466 (1971).
4. Matejovsky, Z. Effects of cytembena in the treatment of malignant musculoskeletal tumours. Neoplasma 18(5): 473-480 (1971).
5. Baker, H., Samson, M., and Izbicki, R. Phase I and II evaluation of cytembena in disseminated epithelial ovarian cancers and sarcomas. Cancer Treat. Rep. 60(9): 1389-1391 (1976).
6. Edmonson, J., Decker, D., Malkasian, G., Webb, M., and Jorgensen, E. Phase II evaluation of cytembena (NSC-104801) in patients with advanced ovarian carcinoma resistant to alkylating agents: brief communication. J. Natl. Cancer Inst. 59(6): 1619-1620 (1977).
7. Falkson, H. and Falkson, G. Phase II trial of cytembena in patients with advanced ovarian and breast cancer. Cancer Treat. Rep. 60(11): 1655-1658 (1976).
8. Armitage, P. Statistical Methods in Medical Research. John Wiley & Sons, Inc., New York, 1971, pp. 362-365.
9. Cox, D. R. Analysis of Binary Data. Methuen & Co., Ltd., London, 1970, pp. 48-52.
10. Gart, J. J. The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39: 148-169 (1971).
11. Tarone, R. E. Tests for trend in life table analysis. Biometrika 62: 697-682 (1975).
12. Sontag, J. M., Page, N. P., and Saffiotti, U. Guidelines for Carcinogen Bioassay in Small Rodents (National Cancer Institute Carcinogenesis Technical Report Series No. 1), DHEW Publication No. 76-801, Washington, D.C., 1976.
13. NCI/NTP. Carcinogenesis Bioassay of Cytembena (TR 207). National Cancer Institute/National Toxicology Program, U.S. Department of Health and Human Services, 1982, 113 pp.